

WHAT IS CLAIMED:

1. An expression cassette comprising a preselected DNA sequence encoding an RNA molecule operably linked to a promoter functional in a host cell,
5 wherein the RNA molecule is substantially complementary to all or a portion of a mRNA encoding a plant seed storage protein.
2. An expression cassette comprising a preselected DNA sequence encoding an RNA molecule operably linked to a promoter functional in a host cell,
10 wherein the RNA molecule is substantially identical to all or a portion of a mRNA encoding a plant seed storage protein.
3. The expression cassette of claim 1 or 2 in which the plant seed storage protein is a maize seed storage protein.
- 15 4. The expression cassette of claim 3 wherein the maize seed storage protein is an α -zein protein.
5. The expression cassette of claim 1 or 2 which further comprises a selectable
20 marker gene.
6. The expression cassette of claim 1 or 2 further comprising plasmid DNA.
7. The expression cassette of claim 1 or 2 wherein the promoter is a promoter
25 functional during plant seed development.

8. The expression cassette of claim 1 wherein the promoter comprises the 10 kD zein promoter.
9. The expression cassette of claim 1 wherein the promoter comprises the 27 kD zein promoter.
10. The expression cassette of claim 1, 8 or 9 wherein the preselected DNA sequence encodes an RNA molecule that is substantially complementary to all or a portion of a mRNA for a 19 kD α -zein protein.
11. The expression cassette of claim 1, 8 or 9 wherein the preselected DNA sequence encodes an RNA molecule that is substantially complementary to all or a portion of a mRNA for a 22 kD α -zein protein.
12. The expression cassette of claim 2, 8 or 9 wherein the preselected DNA sequence encodes an RNA molecule that is substantially identical to all or a portion of a mRNA for a 19 kD α -zein protein.
13. The expression cassette of claim 2, 8 or 9 wherein the preselected DNA sequence encodes an RNA molecule that is substantially identical to all or a portion of a mRNA for a 22 kD α -zein protein.
14. The expression cassette of claim 2 or 9 wherein the preselected DNA sequence encodes MB1.

15. The expression cassette of claim 1 or 2 which further comprises a second preselected DNA sequence which encodes kernel hardness.

5 16. A method for increasing the weight percent of at least one amino acid which is essential to the diet of animals in plant seed, comprising:

10 a) stably transforming plant cells with an expression cassette comprising a preselected DNA sequence encoding an RNA molecule operably linked to a promoter functional in the plant cell so as to yield a transformed plant cell, wherein the RNA molecule is substantially identical, or complementary, to all or a portion of a mRNA encoding a seed storage protein;

15 b) regenerating the transformed cells into a fertile transgenic plant which produces seeds, in which the preselected DNA sequence is expressed in the seeds in an amount sufficient to decrease the weight percent of the seed storage protein relative to the weight percent of the seed storage protein present in seeds of a corresponding nontransgenic plant; and

(c) recovering said transgenic seeds from said transgenic plant.

20 17. A method for increasing the weight percent of starch in a seed, which comprises:

25 a) stably transforming plant cells with an expression cassette comprising a preselected DNA sequence encoding an RNA molecule operably linked to a promoter functional in the plant cell so as to yield transformed plant cells, wherein the RNA molecule is substantially identical, or complementary, to all or a portion of a mRNA encoding a seed storage protein;

- 5 b) regenerating the transformed cell into a fertile transgenic plant which produces seeds in which the preselected DNA sequence is expressed in an amount effective to decrease the production of the seed storage protein, so as to increase the weight percent of starch in the transgenic seed over the weight percent of starch present in the corresponding untransformed seed; and
- (c) recovering said transgenic seeds.
- 10 18. A method for increasing the starch extractability of a seed, which comprises:
- a) stably transforming plant cells with an expression cassette comprising a preselected DNA sequence encoding an RNA molecule operably linked to a promoter functional in the plant cell so as to yield transformed plant cells, wherein the RNA molecule is
- 15 substantially identical, or complementary, to all or a portion of a mRNA encoding a seed storage protein;
- b) regenerating the transformed cell into a fertile transgenic plant which produces seeds in which the preselected DNA sequence is expressed in an amount effective to decrease the production of the seed storage protein, so as to increase the starch extractability of the transgenic seed over the starch extractability of the corresponding
- 20 untransformed seed; and
- (c) recovering said transgenic seeds.
- 25 19. A method for inhibiting the expression of a plant seed storage protein in a plant seed which comprises:
- (a) stably transforming plant cells with an expression cassette

comprising a preselected DNA sequence encoding an RNA molecule operably linked to a promoter functional in a plant cell so as to yield transformed plant cells, wherein the RNA molecule is substantially identical, or complementary, to all or a portion of a messenger RNA for the plant seed storage protein;

(b) regenerating the transformed cells into a fertile transgenic plant which generates plant seeds, wherein the preselected DNA sequence is expressed in the seeds in an amount effective to substantially reduce expression of the plant seed storage protein; and

(c) recovering said seeds.

20. The method of claim 16, 17, 18 or 19 wherein the preselected DNA segment encodes an RNA molecule that is substantially identical to all or a portion of the mRNA encoding a seed storage protein.

21. The method of claim 16, 17, 18 or 19 wherein the preselected DNA segment encodes an RNA molecule that is substantially complementary to all or a portion of the mRNA encoding a seed storage protein.

22. The method of claim 20 wherein the preselected DNA segment encodes an RNA molecule that is substantially identical to all or a portion of the mRNA encoding an α -zein protein.

23. The method of claim 21 wherein the preselected DNA segment encodes an RNA molecule that is substantially complementary to all or a portion of the mRNA encoding an α -zein protein.

24. The method of claim 16, 17, 18 or 19 wherein the plant cell is a monocot cell.
- 5 25. The method of claim 24 wherein the cell is a maize cell.
26. The method of claim 16 or 19 in which the seeds of the transgenic plant have an increased weight percent of at least one essential amino acid.
- 10 27. The method of claim 26 wherein the essential amino acid is selected from the group consisting of methionine, threonine, lysine, tryptophan, isoleucine and mixtures thereof,
28. The method of claim 26 wherein the weight percent of the amino
15 acid is increased at least about 50% to 300%.
29. The method of claim 16, 17, 18 or 19 wherein the preselected DNA sequence is operably linked to a promoter functional during plant seed development.
- 20 30. The method of claim 16, 17, 18 or 19 wherein the promoter comprises the 10 kD zein promoter.
31. The method of claim 16, 17, 18 or 19 wherein the promoter comprises the 27
25 kD zein promoter.

32. The method of claim 21 wherein the preselected DNA sequence encodes an RNA molecule substantially complementary to all or a portion of a mRNA encoding a 19 kD α -zein protein.
- 5 33. The method of claim 21 wherein the preselected DNA sequence encodes an RNA molecule substantially complementary to all or a portion of a messenger RNA encoding a 22 kD α -zein protein.
- 10 34. The method of claim 20 wherein the preselected DNA sequence encodes an RNA molecule substantially identical to all or a portion of a mRNA encoding a 19 kD α -zein protein.
- 15 35. The method of claim 21 wherein the preselected DNA sequence encodes an RNA molecule substantially identical to all or a portion of a messenger RNA encoding a 22 kD α -zein protein.
36. The method of claim 16, 17, 18 or 19 which further comprises stably transforming the cells with a second preselected DNA sequence which encodes kernel hardness.
37. The method of claim 16, 17, 18 or 19 wherein the cell is transformed by a method selected from the group consisting of electroporation, microinjection, microprojectile bombardment, and liposomal encapsulation.

38. The method of claim 16, 17, 18 or 19 which further comprises stably transforming the cells with at least one selectable marker gene.
39. The method of claim 26 further comprising breeding the fertile transgenic plant to yield a progeny plant that has an increase in the weight percent of the at least one amino acid as a dominant trait while still maintaining functional agronomic characteristics relative to the corresponding untransformed plant.
40. The method of claim 17 or 18 further comprising breeding the fertile transgenic plant to yield a progeny plant that has an increase in the weight percent of starch as a dominant trait while still maintaining functional agronomic characteristics relative to the corresponding untransformed plant.
41. A method for inhibiting expression of a family of seed storage protein genes in a plant seed, which comprises:
- (a) stably transforming plant cells with a first preselected DNA sequence which encodes a RNA molecule operably linked to a promoter functional in a plant or seed so as to yield transformed plant cells, wherein the RNA molecule is substantially identical, or complementary, to all or a portion of a messenger RNA that encodes a polypeptide which is substantially homologous in seed storage proteins; and
 - (b) regenerating the transformed cells into a fertile transgenic plant which yields transgenic seeds, wherein the preselected DNA sequence is expressed in the seeds in an amount effective to substantially reduce expression of seed storage proteins in the transgenic seeds relative to the

expression of seed storage proteins in the corresponding nontransgenic seeds.

- 5 42. A method for increasing the weight percent of at least one amino acid essential to the diet of animals in a plant seed, which comprises:
- (a) stably transforming plant cells with a first preselected DNA sequence and a second preselected DNA sequence so as to yield transformed plant cells, wherein the first preselected DNA sequence encodes a RNA molecule substantially identical, or complementary, to all or a portion of a messenger RNA encoding a seed storage protein, wherein the
- 10 second preselected DNA sequence encodes a polypeptide having at least one amino acid essential to the diet of animals, and wherein each preselected DNA sequence is operably linked to a promoter functional in a plant or seed; and
- 15 (b) regenerating the transformed cell into a fertile transgenic plant which yields transgenic seeds, wherein the first preselected DNA sequence is expressed in an amount effective to substantially reduce the production of said seed storage protein in said transgenic seeds relative to the amount of said seed storage protein present in the corresponding nontransgenic seeds,
- 20 and wherein the second preselected DNA sequence is expressed in an amount sufficient to increase the weight percent of the at least one essential amino acid in said transgenic seeds relative to the amount of said essential amino acid present in the corresponding nontransgenic seeds.
- 25 43. A method for increasing the production of a polypeptide in a seed, which comprises:
- (a) stably transforming plant cells with a first preselected DNA

sequence and a second preselected DNA sequence so as to yield a transformed cell, wherein the first preselected DNA sequence encodes an RNA molecule substantially identical, or complementary, to all or a portion of at least one messenger RNA encoding a seed storage protein, wherein the second preselected DNA molecule encodes a polypeptide, and wherein each preselected DNA sequence is operably linked to a promoter functional in said plant; and

(b) regenerating the transformed cells into a fertile transgenic plant which produces transgenic seeds, wherein the first preselected DNA sequence is expressed in the transgenic seeds in an amount effective to substantially reduce the production of the seed storage protein relative to the amount of said seed storage protein present in the corresponding nontransgenic seeds, and wherein the second preselected DNA sequence is expressed in said transgenic seeds as a protein in a weight percent which is substantially increased over the weight percent of that protein in the corresponding nontransgenic seeds.

44. The method of claim 41, 42 or 43 further comprising collecting the transgenic seeds.

45. The method of claim 41 wherein the polypeptide is substantially homologous in α -zein proteins.

46. The method of claim 42 or 43 wherein the first preselected DNA segment encodes an RNA molecule that is substantially identical to all or a portion of the mRNA encoding a seed storage protein.

47. The method of claim 42 or 43 wherein the first preselected DNA segment encodes an RNA molecule that is substantially complementary to all or a portion of the mRNA encoding a seed storage protein.
- 5 48. The method of claim 46 wherein the preselected DNA segment encodes an RNA molecule that is substantially identical to all or a portion of the mRNA encoding an α -zein protein.
- 10 49. The method of claim 47 wherein the preselected DNA segment encodes an RNA molecule that is substantially complementary to all or a portion of the mRNA encoding an α -zein protein.
50. The method of claim 41, 42 or 43 wherein the plant cell is a monocot cell.
- 15 51. The method of claim 50 wherein the cell is a maize cell.
52. The method of claim 42 in which the seeds of the transgenic plant have an increased weight percent of at least one essential amino acid.
- 20 53. The method of claim 52 wherein the essential amino acid is selected from the group consisting of methionine, threonine, lysine, tryptophan, isoleucine and mixtures thereof.
- 25 54. The method of claim 52 wherein the weight percent of the amino

acid is increased at least about 50% to 300%.

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55. The method of claim 41, 42 or 43 wherein the preselected DNA sequence is operably linked to a promoter functional during plant seed development.
56. The method of claim 41, 42 or 43 wherein the promoter comprises the 10 kD zein promoter.
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57. The method of claim 41, 42 or 43 wherein the promoter comprises the 27 kD zein promoter.
58. The method of claim 49 wherein the preselected DNA sequence encodes an RNA molecule substantially complementary to all or a portion of a mRNA encoding a 19 kD α -zein protein.
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59. The method of claim 49 wherein the preselected DNA sequence encodes an RNA molecule substantially complementary to all or a portion of a messenger RNA encoding a 22 kD α -zein protein.
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60. The method of claim 48 wherein the preselected DNA sequence encodes an RNA molecule substantially identical to all or a portion of a mRNA encoding a 19 kD α -zein protein.
61. The method of claim 48 wherein the preselected DNA sequence

encodes an RNA molecule substantially identical to all or a portion of a messenger RNA encoding a 22 kD α -zein protein.

- 5 62. The method of claim 42 or 43 wherein the second preselected DNA sequence encodes MB1.
63. The method of claim 42 or 43 wherein the second preselected DNA sequence encodes a 10 kD zein.
- 10 64. The method of claim 42 or 43 wherein the cells are stably transformed with a third preselected DNA sequence which encodes kernel hardness.
65. The method of claim 64 wherein the third preselected DNA sequence encodes a 27 kD zein protein.
- 15 66. The method of claim 41 wherein the cells are stably transformed with a second preselected DNA sequence which encodes kernel hardness.
- 20 67. The method of claim 66 wherein the second preselected DNA sequence encodes a 27 kD zein protein.
- 25 68. The method of claim 41, 42 or 43 wherein the cell is transformed by a method selected from the group consisting of electroporation, microinjection, microprojectile bombardment, and liposomal encapsulation.

69. The method of claim 41, 42 or 43 wherein the cells are stably transformed with at least one selectable marker gene.

5 70. The method of claim 42 further comprising breeding the fertile transgenic plant to yield a progeny plant that has an increase in the weight percent of the at least one amino acid as a dominant trait while still maintaining functional agronomic characteristics relative to the corresponding untransformed plant.

10 71. A fertile transgenic *Zea mays* plant having an increased weight percent of at least one amino acid which is essential to the diet of an animal, the genome of which is stably augmented by a preselected DNA sequence encoding an RNA molecule which is substantially identical, or complementary, to a mRNA encoding a plant seed storage protein, wherein the preselected DNA
15 sequence is expressed in the cells of the transgenic plant in an amount sufficient to decrease the amount of the seed storage protein relative to the amount of said seed storage protein in the cells of a plant which only differ from the cells of said transgenic plant in that said preselected DNA sequence is absent, and wherein said preselected DNA sequence is transmitted through
20 a complete normal sexual cycle of the transgenic plant to the next generation.

25 72. A fertile transgenic *Zea mays* plant having an increased starch content, the genome of which is stably augmented by a preselected DNA sequence encoding an RNA molecule which is substantially identical, or complementary, to a mRNA encoding a plant seed storage protein, wherein the preselected DNA sequence is expressed in the cells of the transgenic

plant in an amount sufficient to decrease the amount of said seed storage protein and to increase the starch content relative to the amount of said seed storage protein and starch content in the cells of a plant which only differ from the cells of said transgenic plant in that said preselected DNA sequence is absent, and wherein said preselected DNA sequence is transmitted through a complete normal sexual cycle of the transgenic plant to the next generation.

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73. A fertile transgenic *Zea mays* plant, the seeds of which have an increased starch extractability, the genome of said plant which is stably augmented by a preselected DNA sequence encoding an RNA molecule which is substantially identical, or complementary, to a mRNA encoding a plant seed storage protein, wherein the preselected DNA sequence is expressed in the seeds of the transgenic plant in an amount sufficient to decrease the amount of said seed storage protein and to increase the starch extractability of the seed relative to the amount of said seed storage protein and starch extractability in the seeds of a plant which only differ from the seeds of said transgenic plant in that said preselected DNA sequence is absent, and wherein said preselected DNA sequence is transmitted through a complete normal sexual cycle of the transgenic plant to the next generation.

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74. A fertile transgenic *Zea mays* plant having a decreased amount of seed storage protein, the genome of which is stably augmented by a preselected DNA sequence encoding an RNA molecule which is substantially identical, or complementary, to a mRNA encoding a seed storage protein, wherein the RNA molecule is substantially identical, or complementary, to all or a portion of a mRNA molecule that encodes a peptide that is substantially homologous in seed storage proteins, wherein the preselected DNA sequence

is expressed in the cells of the transgenic plant in an amount sufficient to decrease the amount of seed storage proteins in the cells of a plant which only differ from the cells of said transgenic plant in that said preselected DNA sequence is absent, and wherein said preselected DNA sequence is transmitted through a complete normal sexual cycle of the transgenic plant to the next generation.

75. A fertile transgenic *Zea mays* plant having a decreased seed storage protein content, the genome of which is stably augmented by a preselected DNA sequence encoding an RNA molecule which is substantially identical, or complementary, to a mRNA encoding a seed storage protein, wherein the preselected DNA sequence is expressed in the cells of the transgenic plant in an amount sufficient to decrease the amount of said protein in the cells of a plant which only differ from the cells of said transgenic plant in that said preselected DNA sequence is absent, and wherein said preselected DNA sequence is transmitted through a complete normal sexual cycle of the transgenic plant to the next generation.
76. A fertile transgenic *Zea mays* plant having an increased weight percent of at least one amino acid essential to the diet of an animal, the genome of which is stably augmented by a first preselected DNA sequence and a second preselected DNA sequence, wherein the first preselected DNA sequence encodes an RNA molecule which is substantially identical, or complementary, to a mRNA encoding a seed storage protein, wherein the second preselected DNA sequence encodes a polypeptide having at least one amino acid essential to the diet of an animal, wherein the first preselected DNA sequence is expressed in the cells of the transgenic plant in an amount sufficient to decrease the amount of said seed storage protein and the second

preselected DNA sequence is expressed in the cells of the transgenic plant in an amount sufficient to increase the weight percent of the at least one amino acid essential to the diet of an animal relative to the amount of said seed storage protein and weight percent of said essential amino acid in the cells of a plant which only differ from the cells of said transgenic plant in that said preselected DNA sequences are absent, and wherein said preselected DNA sequences are transmitted through a complete normal sexual cycle of the transgenic plant to the next generation.

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- 10 77. A fertile transgenic *Zea mays* plant having an increased amount of a preselected polypeptide, the genome of which is stably augmented by a first preselected DNA sequence and a second preselected DNA sequence, wherein the first preselected DNA sequence encodes an RNA molecule which is substantially identical, or complementary, to a mRNA encoding a
- 15 seed storage protein, wherein the second preselected DNA sequence encodes a preselected polypeptide, wherein the first preselected DNA sequence is expressed in the cells of the transgenic plant in an amount sufficient to decrease the amount of said seed storage protein and the second preselected DNA sequence is expressed in the cells of the transgenic plant in an amount
- 20 sufficient to increase the amount of said preselected polypeptide relative to the amount of said seed storage protein and said preselected polypeptide in the cells of a plant which only differ from the cells of said transgenic plant in that said preselected DNA sequences are absent, and wherein said preselected DNA sequences are transmitted through a complete normal
- 25 sexual cycle of the transgenic plant to the next generation.

78. A seed derived from the plant of claim 71, 72, 73, 74, 75, 76 or 77.

79. A progeny plant derived from the seed of claim 78.

80. The transgenic plant of claim 74 wherein the preselected DNA sequence encodes an RNA molecule which is substantially identical, or complementary, to all or a portion of an mRNA that encodes a peptide that is substantially homologous in α -zein proteins.

81. The transgenic plant of claim 71 or 75 in which the seeds of the transgenic plant have an increased weight percent of at least one essential amino acid.

82. The transgenic plant of claim 81 wherein the essential amino acid is selected from the group consisting of methionine, threonine, lysine, tryptophan, isoleucine and mixtures thereof.

83. The transgenic plant of claim 81 wherein the weight percent of the amino acid is increased at least about 50% to 300%.

84. The transgenic plant of claim 71, 72, 73, 74, or 75 wherein the promoter comprises the 10 kD zein promoter.

85. The transgenic plant of claim 76 or 77 wherein at least one promoter comprises the 10 kD zein promoter.

86. The transgenic plant of claim 71, 72, 73, 74 or 75 wherein the promoter comprises the 27 kD zein promoter.

87. The transgenic plant of claim 76 or 77 wherein at least one promoter comprises the 27 kD zein promoter.
- 5 88. The transgenic plant of claim 71, 72, 73, 74, 75, 76 or 77 wherein the preselected DNA sequence, which encodes an RNA molecule substantially complementary to all or a portion of a mRNA encoding a seed storage protein, encodes an RNA molecule substantially complementary to all or a portion of a mRNA encoding 19 kD α -zein protein.
- 10 89. The transgenic plant of claim 71, 72, 73, 74, 75, 76 or 77 wherein the preselected DNA sequence, which encodes an RNA molecule substantially complementary to all or a portion of a mRNA encoding a seed storage protein, encodes an RNA molecule substantially complementary to all or a portion of a mRNA encoding a 22 kD α -zein protein.
- 15 90. The transgenic plant of claim 71, 72, 73, 74, 75, 76 or 77 wherein the preselected DNA sequence, which encodes an RNA molecule substantially identical to all or a portion of a mRNA encoding a seed storage protein, encodes an RNA molecule substantially identical to all or a portion of a mRNA encoding a 19 kD α -zein protein.
- 20 91. The transgenic plant of claim 71, 72, 73, 74, 75, 76 or 77 wherein the preselected DNA sequence, which encodes an RNA molecule substantially identical to all or a portion of a mRNA encoding a seed storage protein, encodes an RNA molecule substantially identical to all or a portion of a mRNA encoding a 22 kD α -zein protein.
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92. The transgenic plant of claim 76 or 77 wherein the second preselected DNA sequence encodes MB1.

5 93. The transgenic plant of claim 76 or 77 wherein the second preselected DNA sequence encodes a 10 kD zein.

94. The transgenic plant of claim 71, 72, 73, 74, 75, 76 or 77 further comprising stably transforming the cells with a gene which encodes kernel hardness.

10 95. The transgenic plant of claim 71, 72, 73, 74, 75, 76 or 77 wherein the cell is transformed by a method selected from the group consisting of electroporation, microinjection, microprojectile bombardment, and liposomal encapsulation.

15 100. The transgenic plant of claim 75, 76, 77, 78, 79, 80 or 81 further comprising stably transforming the cells with at least one selectable marker gene.

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